Hemopoiesis in Pituitary Dwarfs Treated With Human Growth Hormone and Testosterone

By J. H. Jepson and E. E. McGarry

Prepubertal hypopituitary dwarfs are anemic and have reduced levels of erythropoietin-stimulating factor (ESF) in the urine. Treatment with human growth hormone (HGH) in doses sufficient to produce linear growth and weight gain induces bone marrow lymphocytosis, increased erythropoiesis indicated by an increase of the red cell mass (RCM) and improved iron kinetic studies. This was associated with increased concentrations of ESF in the urine, increased transferrin levels, and expansion of the plasma volume. The combination of testosterone and HGH increased further erythropoiesis but could not be specifically ascribed to testosterone, since the continued action of HGH could be responsible for the observed effects. The excretion of ESF was not increased further. Testosterone did, however, maintain ESF excretion on deletion of HGH from the regimen, suggesting at least one common mode of action for these hormones in the hypopituitary subject. In contrast, testosterone failed to maintain the plasma volume increase induced by HGH and did not further effect the observed bone marrow lymphocytosis.

Prepubertal dwarfs, deficient in both growth hormone and testosterone have reduced erythrocyte values.1-6 We have studied the anemia of a group of these and the response to treatment with human growth hormone (HGH) and testosterone. These studies indicate that in human pituitary dwarfs anemia is associated with low excretion of erythropoietin-stimulating factor (ESF) in a range similar to that of prepubertal subjects and adult females. The data to be reported indicates that both these hormones could play an important role in the increase of erythrocyte values observed during normal growth and sexual maturation.

CASE REPORT

Panhypopituitarism in this group of patients was either idiopathic or followed surgical intervention for craniopharyngioma. Subjects were classified as prepubertal by chronologic age, bone age, absence of secondary sexual characteristics, and endocrinologic determinations.

All subjects were infantile proportionate dwarfs with normal chromosomal idiograms, psychological stability, and intelligence. Growth hormone deficiency was determined by measuring serum GH levels following insulin hypoglycemia and showed the characteristic

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response to HGH administration. All were shown to have deficiencies of TSH, ACTH, and FSH by standard procedures. Other possible or contributing etiologies of growth retardation were also excluded.

All were on maintenance endocrine therapy of thyroid hormone and adrenal steroids unless otherwise noted. In the following text maintenance therapy refers to that received prior to the addition of HGH and testosterone. Two and a half milligrams of intramuscular HGH (Nordic Biochemical, Upsala, Sweden) were given thrice weekly. In some cases, to attain maximum growth, this was increased to 2.5-5 mg daily. When male subjects had attained sufficient height on this regimen, 200 mg of intramuscular testosterone enanthate were injected every second week. This was sufficient to induce secondary sexual characteristics.

Platelets of these patients, evaluated by examining the peripheral blood smear, were abundant prior to and during therapy. The red cell mass, erythrocyte survival, and iron-kinetic studies were carried out prior to, and at various intervals after, the addition of HGH and later upon the addition of testosterone.

Normal prepubertal controls were patients with constitutional small stature who had been admitted to the metabolic ward for investigation. Additional erythropoietin studies were carried out on prepubertal subjects in the same weight range with normal endocrine status.

MATERIALS AND METHODS

Routine hematologic, biochemical, and endocrine studies were done employing standard laboratory procedures. Bone marrow specimens, obtained from the posterior iliac crest, were stained with Jenner and Giemsa and for iron according to the method of Finch.7 The myeloid:erythroid (M:E) ratio was calculated from counting 200 cells. The total blood volume and red cell mass were measured employing radioiodinated serum albumin8 and 51Cr-labeled erythrocyte dilution techniques.9 Survival of 51Cr-labeled erythrocytes10 and iron-kinetic studies11 were also determined.

Twenty-four-hour urines were collected, concentrated, and stored until use as described previously.3,12 The erythropoietic content of these urine specimens was determined in polycythemic mice,12 and the number of units per specimen calculated as described previously.13

The concentration of HGH in urine specimens of HGH-treated subjects has previously been shown to be less than 0.1 μg/liter by radioimmunoassay.14 In order to insure that HGH did not augment the erythropoietic effect of ESF contained in the specimen, 30 μg of HGH were injected alone and in combination with ESF as described previously.15

RESULTS

Peripheral Hematologic Values

The mean hemoglobin concentration, reticulocyte count, and red cell mass (RCM) of pituitary-deficient dwarfs on maintenance therapy on admission to the hospital were significantly (at 2 SEM, p<0.05) lower than those of prepubertal normal subjects, but the mean plasma volume was not. Human growth hormone significantly increased all of these parameters over that of subjects on maintenance therapy. The RCM was increased by 30% into the low normal range. On a combination of HGH and testosterone the mean hemoglobin and reticulocyte counts continued to increase, but neither the mean red cell mass nor plasma volume was significantly increased over the growth hormone treated group (Table 1). In both treated groups a reticulocytosis was observed early in the course of administration (Figs. 1, 2). The continued sequential increase of hematologic parameters on this combination is shown in Fig. 1. When HGH was deleted from the regimen, the RCM decreased from 28.2 to 23.5
ml/kg despite further weight gain and sexual maturation. A highly significant decrease of the plasma volume (PV) also occurred, resulted in a false rise of the hemoglobin concentration.

All patients tended to have a neutropenic leukopenia at times cyclic and not altered significantly by injection of HGH or testosterone (Figs. 1, 2, 4). Peripheral lymphocyte counts were all within the normal range. The \( t^{1/2} \) survival of autologous \(^{51}\)Cr-labeled erythrocytes was normal in untreated and treated groups (23–31 days).

**Bone Marrow**

The bone marrow of patients who were receiving maintenance therapy showed normal cellularity with an M:E ratio of 3:1. Erythropoiesis was

<table>
<thead>
<tr>
<th>Hypopituitary Therapy</th>
<th>Hemoglobin (g/100 ml)</th>
<th>Reticulocytes (%)</th>
<th>Red Cell Mass (ml/kg)</th>
<th>Plasma Volume (ml/kg)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance*</td>
<td>12.0 ± 0.14†</td>
<td>0.5 ± 0.12</td>
<td>17.7 ± 0.93</td>
<td>44.8 ± 3.4</td>
<td>Compared to normal prepubertal subjects</td>
</tr>
<tr>
<td></td>
<td>(11-13)</td>
<td>(0.1-1.4)</td>
<td>(14-19.2)</td>
<td>(38.5-54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(( p &lt; 0.001 ))</td>
<td>(( p &lt; 0.001 ))</td>
<td>(( p &lt; 0.001 ))</td>
<td>(( p &lt; 0.4 ))</td>
<td></td>
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<tr>
<td>+ HGH</td>
<td>13.4 ± 0.2</td>
<td>2.5 ± 0.24</td>
<td>23.3 ± 0.78</td>
<td>50.4 ± 1.02</td>
<td>Compared to maintenance subjects</td>
</tr>
<tr>
<td></td>
<td>(12.6-14.9)</td>
<td>(1-4)</td>
<td>(19.3-28.2)</td>
<td>(41-61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(( p &lt; 0.001 ))</td>
<td>(( p &lt; 0.001 ))</td>
<td>(( p &lt; 0.001 ))</td>
<td>(( p &lt; 0.001 ))</td>
<td></td>
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<tr>
<td>+ Testosterone</td>
<td>14.9 ± 0.39</td>
<td>3.3 ± 0.48</td>
<td>26.3 ± 1.11</td>
<td>49.4 ± 2.4</td>
<td>Compared to HGH-treated subjects</td>
</tr>
<tr>
<td></td>
<td>(14-15.6)</td>
<td>(1.5-5.6)</td>
<td>(23-28.2)</td>
<td>(41-61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(( p &lt; 0.05 ))</td>
<td>(( p &lt; 0.001 ))</td>
<td>(( p &lt; 0.1 ))</td>
<td>(( p &lt; 0.3 ))</td>
<td></td>
</tr>
<tr>
<td>Normal prepubertal</td>
<td>13.4 ± 0.17</td>
<td>1.5 ± 0.37</td>
<td>25.3 ± 1.23</td>
<td>41.4 ± 1.35</td>
<td></td>
</tr>
<tr>
<td>subjects</td>
<td>(12.5-14.2)</td>
<td>(0.5-2.8)</td>
<td></td>
<td></td>
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</table>

\( * \)Compared to normal prepubertal subjects.

\( † \)Standard error of mean.
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Fig. 2. Clinical course of prepubertal female pituitary dwarf prior to and during HGH therapy. Lower graph: open circles, plasma volume (PV); black circles, red cell mass (RCM); black diamonds, concentration of erythropoietin (ESF) in 24 hr urine.

normoblastic, and megakaryopoiesis was normal. Lymphocytes constituted 20%-25% of the total marrow count.

During administration of HGH, erythropoiesis appeared normal to increased as the dose of HGH was increased. The mean M:E ratio was 2.1. The most striking change was an increase of the number of lymphocytes to 35%-45% of the total number of bone marrow cells.

When testosterone was added to the regimen, the total number of lymphocytes did not change, and the M:E ratio was 1.9:1. Granulopoiesis was qualitatively normal in these treated groups, with a relative reduction in number due to the increase of lymphocytes. Megakaryopoiesis was both qualitatively and quantitatively normal. Hemosiderin deposition was normal in all cases.

Iron Kinetics

In pituitary dwarfs on maintenance therapy the mean serum iron ($p < 0.0025$) total transferrin concentration ($p < 0.005$) and $t^{1/2}$ clearance time of iron from plasma ($p < 0.0025$) were significantly decreased below that of normal prepubertal subjects, while the mean plasma iron turnover rate was within the normal range (Table 2).

On injection of HGH the mean serum iron ($p < 0.005$) and total transferrin concentration ($p < 0.001$) were increased significantly over that of pituitary dwarfs on maintenance therapy. The mean $t^{1/2}$ clearance time of radioiron from plasma was significantly more rapid ($p < 0.001$) than that of the mean of normal subjects but not significantly more rapid than those on maintenance therapy alone ($p < 0.1$). The mean 24-hr plasma iron turnover rate (PIT) was increased significantly over that of subjects on maintenance therapy alone ($p < 0.025$), falling at the upper limits of the range for normal subjects.

The mean serum iron and transferrin concentration of subjects receiving HGH and testosterone were not significantly changed ($p < 0.1$). The mean plasma $t^{1/2}$ clearance time ($p < 0.0025$) of radioiron and the mean plasma iron turnover rate of three subjects were increased ($p < 0.05$). However, the range of the PIT was identical to that of subjects receiving only HGH, and there was
Fig. 3. Total concentration of erythropoietin (ESF) in 24-hr urine specimen collected from normal prepubertal and prepubertal pituitary dwarfs with and without HGH and testosterone added to the regimen.

a very small spread of the t½ clearance times. The smaller number of subjects in this group limits the significance of the mean of the group.

The incorporation of ⁵⁹Fe into erythrocytes was within normal limits in all groups (Table 2).

Excretion of Erythropoietin (ESF)
The mean concentration of ESF in urine concentrates of normal male and female prepubertal subjects was slightly less than 1.0 U/24 hr (0.55–1.85 U); that of pituitary dwarfs on maintenance therapy was in the same range with no apparent differences between male and female subjects. On addition of HGH to the therapeutic regimen, the mean excretion of ESF in 24 hr tripled to

<table>
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<tr>
<th>Table 2. Iron Metabolism in Prepubertal Pituitary-deficient Dwarfs Prior to and During Human Growth Hormone and Testosterone Therapy</th>
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<tbody>
<tr>
<td>Normal Prepubertal</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Serum iron (µg/100 ml)</td>
</tr>
<tr>
<td>Total transferrin (µg/100 ml)</td>
</tr>
<tr>
<td>T½ clearance of ⁵⁹Fe (min)</td>
</tr>
<tr>
<td>Plasma iron turnover (mg/kg per day)</td>
</tr>
<tr>
<td>Maximum percentage incorporation of ⁵⁹Fe RBC</td>
</tr>
</tbody>
</table>

*Standard error of mean.
†Numbers in brackets indicate number of patients studied.
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Fig. 4. Clinical course of prepubertal male pituitary dwarf receiving increasing doses of HGH. Lower graph: open circles, plasma volume (PV); black circles, red cell mass (RCM); black diamonds, concentration of erythropoietin (ESF) in 24-hr urine.

3.1 U. The mean excretion of ESF on addition of testosterone did not increase further and was similar to that of the adult male (Fig. 3). There was no correlation of the concentration of ESF in the urine with the RCM/kg of body weight. When the 24-hr excretion of ESF was related to the body weight, the ESF concentration tended to increase as the body weight increased. This was not the case in normal adult females and males. In these subjects there was a significant difference between the sexes (Table 3).

Effect of HGH Alone and Combined With ESF on Erythropoiesis of Polycythemic Mice

In normal humans and pituitary dwarfs treated with HGH no significant amount of HGH is detected in the urine.\textsuperscript{14} In addition, 30\textsuperscript{g} of HGH, which is at least 300 times that found in urine of HGH-treated pituitary dwarfs, neither stimulated erythropoiesis in polycythemic mice nor augmented the erythropoietic effect of ESF.
Table 3. Effect of HGH and ESF, Alone, and in Combination, on Incorporation of 

\[ {^{59}Fe} \text{into Erythrocytes of Polycythemic Mice} \]

<table>
<thead>
<tr>
<th>Test Material and Total Dose</th>
<th>%(^{59}\text{Fe}} \text{RBC} \text{ (Mean ± SE)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGH 30 ( \mu \text{g} )</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>ESF 0.125 U</td>
<td>5.61 ± 0.63</td>
</tr>
<tr>
<td>ESF 0.5 U</td>
<td>17.67 ± 0.73</td>
</tr>
<tr>
<td>HGH 30 ( \mu \text{g} )</td>
<td>+</td>
</tr>
<tr>
<td>+ 0.125 U ESF</td>
<td>(10)</td>
</tr>
<tr>
<td>HGH 30 ( \mu \text{g} )</td>
<td>+</td>
</tr>
<tr>
<td>+ 0.5 U ESF</td>
<td>(12)</td>
</tr>
<tr>
<td>Saline control</td>
<td>0.63 ± 0.1</td>
</tr>
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</table>

\*0.5 Umg ovine ESF, Connaught Laboratories, Toronto, Canada.
†Numbers in parentheses indicate mice per group.

Representative Clinical Course of Treated Subjects

Figure 1: A progressive increase of the body weight, height, RCM, PV, and concentration of ESF in the urine occurred on administration of HGH. On 2.5 mg of HGH thrice weekly increased growth and an increase of the hemoglobin concentration occurred. When the dose was increased to 5 mg/day a reticulocytosis occurred, but the hemoglobin decreased due to concomitant expansion of the plasma volume. Direct measurement of the RCM demonstrated a significant increase and was subsequently reflected by a further increase of the hemoglobin concentration. The excretion of ESF increased significantly over that of non-HGH treated subjects. On addition of testosterone a further reticulocytosis occurred. The RCM continued to increase with no change in the concentration of erythropoietin in the urine. A temporary decline of reticulocytes occurred on deletion of HGH from the regimen with a slight decrease of the RCM and a marked decrease of the plasma volume. However, erythropoietin excretion was maintained at the same level. In this patient the growth rate declined after deletion of HGH, although the weight increase continued and secondary male characteristics developed.

Figure 2: A sexually immature female given HGH resulted in an increase of the reticulocyte count, RCM, and PV. The significant rise of the urine ESF concentration was greater than that found in urine of adult females. The RCM and PV appeared to level off despite a continued linear growth and increase of body weight.

Figure 4: As the dose of HGH was increased in this patient, there was an initial rise of the urine ESF concentration followed by an increase of the RCM and PV despite the slowed growth and weight gain. A fall of the hemoglobin...
concentration, hematocrit, and WBC count appeared to be a reflection of the expansion of the PV.

DISCUSSION

The effect of growth hormone of lower species on erythropoiesis of the rodent has been extensively studied and summarized by several authors.\textsuperscript{16-18} It was generally concluded that the erythropoietic response was due to its anabolic action and that the complete repair of the anemia required a combination of hormones of the anterior pituitary. Few similar or in depth studies of this anemia or its response to hormone therapy have been done in the pituitary deficient human.\textsuperscript{16} Growth hormone failed to stimulate erythropoiesis in plethoric mice or in intact rabbits, and no increase of ESF was found in the plasma.\textsuperscript{19,20} In the acromegalic human, neither an increase of hemoglobin concentration nor excretion of ESF in urine was observed.\textsuperscript{1} Thus previous data suggest that HGH, at least in part, exerts its erythropoietic effect only in hypoanabolic subjects through its general anabolic action. The well-known anabolic action of HGH \textsuperscript{21-23} was perhaps reflected by the increase of transferrin concentration. With an increase in metabolic activity more hemoglobin would be required to deliver oxygen to tissues. The relationship of increased hemoglobin concentration to the proportional increase of total muscle or lean body mass that occurs with growth must be considered.\textsuperscript{24}

A marked increase of plasma volume also occurred by an unknown mechanism and is similar to that produced by a structurally related hormone, placental lactogen.\textsuperscript{25-27}

The significant increase of bone marrow lymphocytes following HGH is also of great interest in view of the hypothesis that this cell could be a pluripotential precursor capable of differentiating into morphologically distinguishable cell lines.\textsuperscript{28} If true, one of the actions of HGH may be on this pluripotential stem cell compartment. This could account for the stimulation of erythropoiesis by growth hormone in nephrectomized, hypophysectomized rats\textsuperscript{29} and increased erythropoiesis in bone marrow perfused in vivo with solutions containing growth hormone.\textsuperscript{30}

A little more is known about the effect of testosterone, its analogues and derivatives, on the synthesis of specific enzymes in the kidney,\textsuperscript{31} on the synthesis of delta-aminolevulinic acid synthetase in chick hemopoietic tissues,\textsuperscript{32} nucleoprotein and heme synthesis in human bone marrow cultures,\textsuperscript{33,34} and (in pharmacologic doses) on the excretion of ESF in the urine of humans.\textsuperscript{5,36} In the present experiments employing physiologic doses, no further knowledge is added to the fundamental effects previously described. Results obtained on addition of testosterone to the therapeutic regimen could be due to the continued effect of HGH on erythropoiesis. The RCM declined slightly, and the plasma volume significantly when HGH was deleted from the therapeutic regimen. However, testosterone appeared to maintain the increased level of ESF in urine. The transferrin concentration increase induced by HGH was not increased further. No effect on bone marrow lymphocytes was observed. The data suggest that in the hypoanabolic subject testosterone could exert hema-
tologic effects similar to that of HGH in terms of its anabolic action but that there is a dichotomy of effect in terms of other actions of these hormones.

APPENDIX

Figure 1

A.T. (male) was first seen in July, 1965. His chronologic age was 18½ yr, with a height age of 10½ yr, and a bone age of 14 yr. Human chorionic gonadotropin (HCG) injections for 6 mo at the age of 14 had resulted in some growth, but induced pubertal changes had receded when treatment was discontinued. He had not grown for 2 yr. He showed laboratory evidence of GH, TSH, and ACTH deficiency. FSH was negative on several occasions. He grew ¾ in on HGH, 2.5 mg three times weekly, over a 4-mo period. Between September, 1966 to May, 1967, HGH was increased to 5 mg/day, and by March 1967 he had grown 2½ in. At this time testosterone enanthate was started, and by May, 1967 he had grown another 1½ in. His entire clinical course on therapy is shown in Fig. 1.

Figure 2

C.T. (female) was first seen in July, 1965 at the age of 12½ yr, with a height age of 7 yr and a bone age of 8 yr. She showed laboratory evidence of GH, ACTH, and TSH deficiency, and the FSH was negative. On no treatment other than L-thyroxine, .2 g/day, she grew ¾ inch in 10 mo. On a balanced diet in the hospital she then grew ¾ inch over a 4-mo period. HGH, 2.5 mg three times weekly, was then given over a 16-mo period, and she grew 3¼ inch. Her clinical course is shown in Fig. 2.

Figure 4

F.H. (male) was initially seen in 1966 at 17¾ yr. His height age was 17¾ yr, and the bone age 11½ yr. At the age of 15 yr he had received orotestin for 1 yr. On his admission to the Royal Victoria Hospital he showed laboratory evidence of FSH and GH deficiency. Although base-line levels of 17 ketogenic steroids were low (2.2 mg/24 hr. sq m), he responded normally to the metapyrone test. FSH was negative. Prior to therapy the hemoglobin was 10.5–11.4 g/100 ml, and the hematocrit 35%–37%. A persistent moderate neutropenia was present with normal lymphocytes. A reticulocytosis (3.2%) was observed following a short course of prolactin. He was discharged on 0.2 mg/day of L-thyroxine and 2.5 mg of HGH three times a week. Over a 13-mo period he grew 2½ inches. In 1967, he was readmitted for 5 mo. In the hospital, on HGH, 2.5 mg three times weekly, and a high protein diet he grew another 2½ inches. In the subsequent 6 mo on HGH, he grew an additional 2½ inches. His clinical course is shown in Fig. 4.

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